

2024-09-22 05:00 UTC



FIG. 1A

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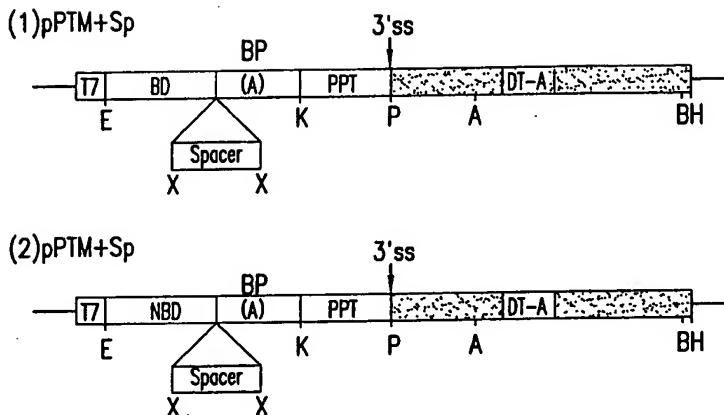


FIG.1B

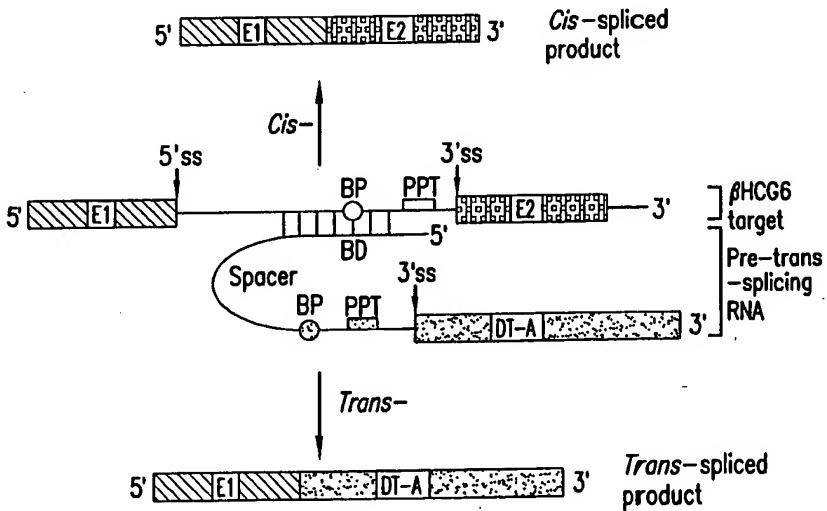
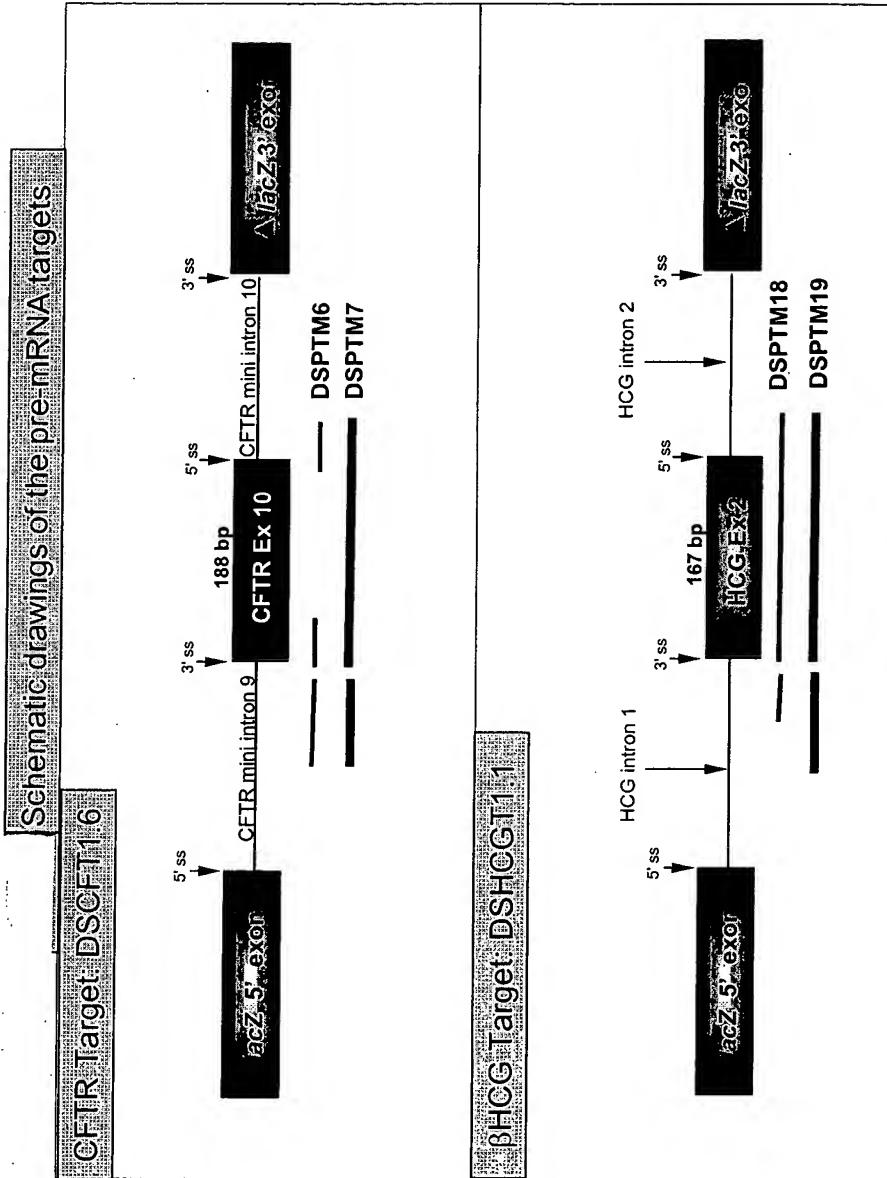


FIG.1C

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Figure 2

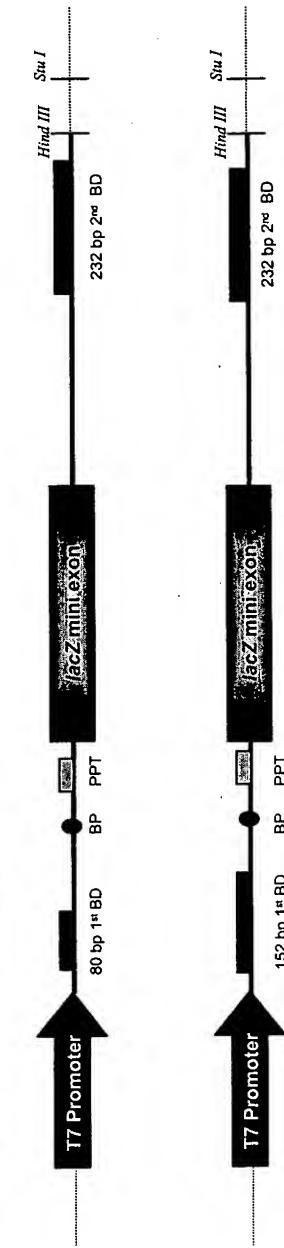
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### Schematic diagrams of double *trans*-splicing PTMs

#### DSP-PTM 6 & 7 (CE-IR Targeted)



#### DSP-PTM 8 & 19 (BHCC Targeted)

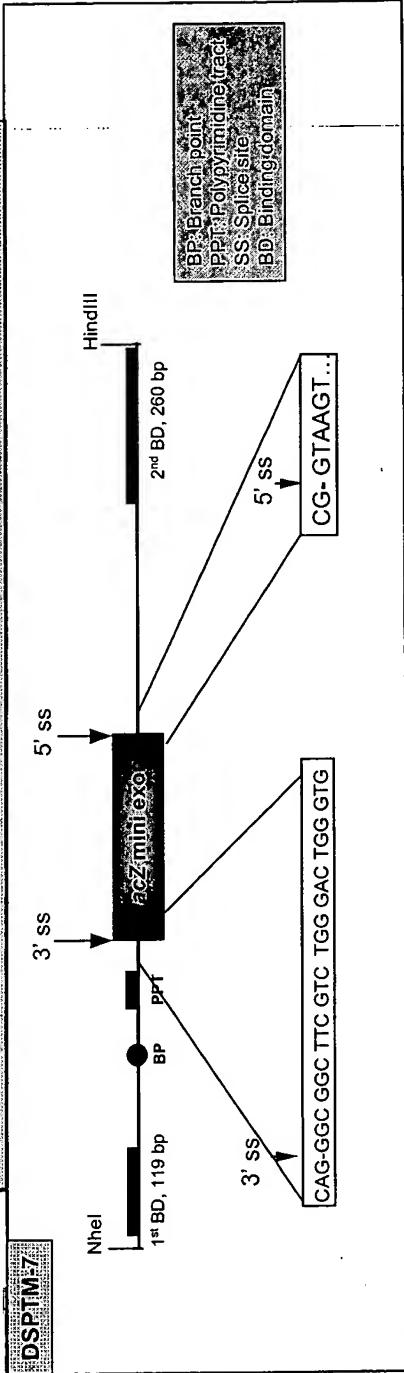


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Figure 3

### Diagram and important structural elements of double trans-splicing PTM7



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**1<sup>st</sup> BD (119 bp):** GATTCACTTGCCTCCAATTATCATCCTAACAGCAGAAAGTGTATATTCTATTGGATAAGATTCTATTAACTCATTGATTCAAATA  
TTTAAAATFACTTCCCTGTTCTACTCTGCTATGCAC

**Spacer sequences:** AACATTATTATAACGTTGCTCGAA

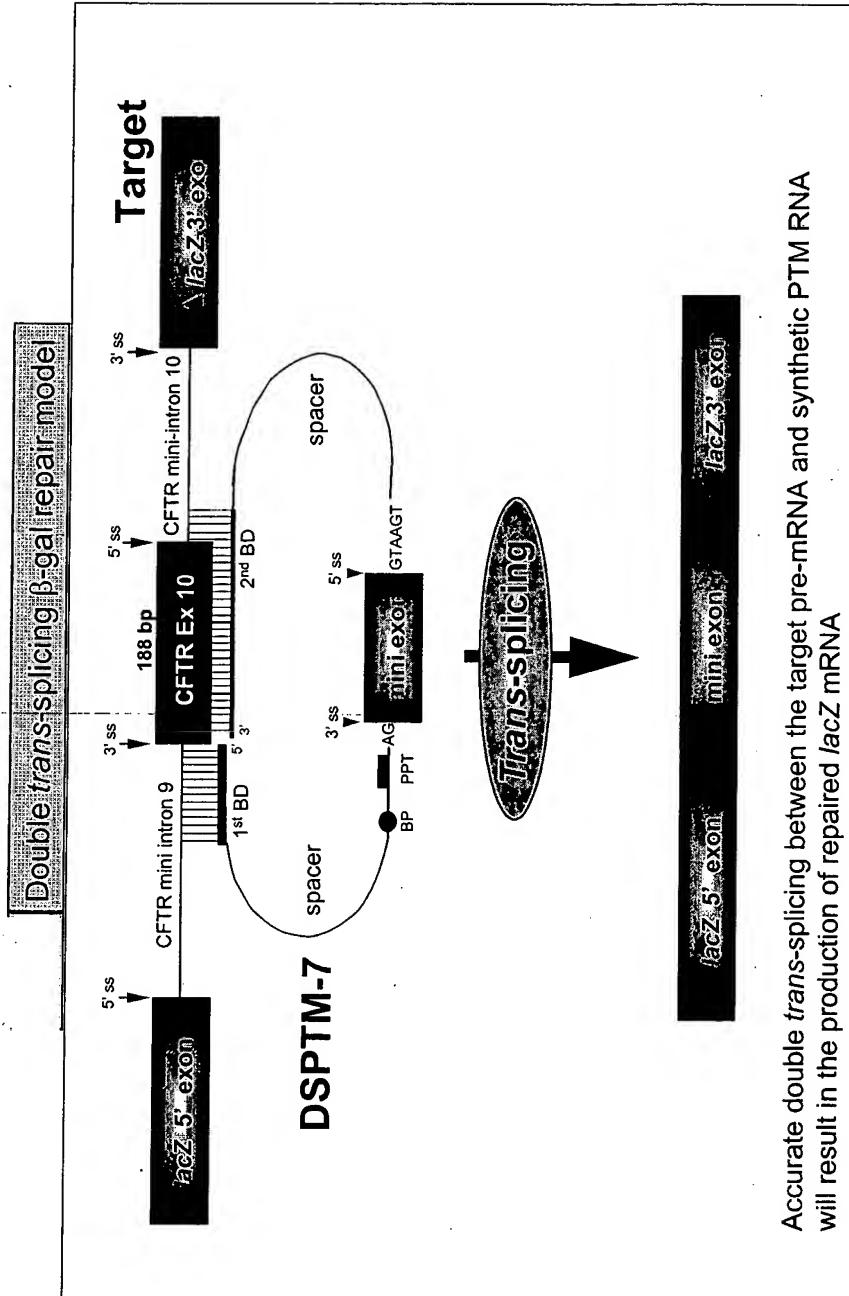
**BP, PPT and acceptor splice site:** TACTAAC T GGTAACC TCTTCTTTTTTTT GATATC CTGCAG GGC GGC TTC GTC TGG GAC TGG

**5' donor site and 2<sup>nd</sup> spacer sequence:** TGA ACG GTAAAGT GTTATCACCGATACTGTC AACCTGATTCTGGCTTCATAACGCTAA  
GATCCACCGG

**2<sup>nd</sup> BD (260 bp):** TCAAAAAGTTTACACATAATTCTTACCTCTCTGAAATTCAATGCTTGTGATGACGCTTCTGTATCTATATCATCTATTGAA  
ACACAATGATTCTTAAATGGTGCCTGGCATAACTCTGGAAACTGATAACACAATGAAATTCTTCCACTGTGCTTAA  
AAAACCCCTCTGAATTCTCCATTCTCCATAATCATCATTACAACGCTGGAAATAAAACCCATCAATTAACTCA  
TTATCAAATCAGCG

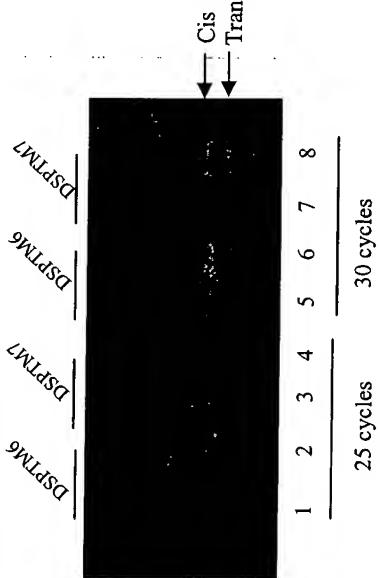
Figure 4

Figure 5



## Proof-of-principle of SMaRT using synthetic double splicing PTM RNA in 293T cells

### 2<sup>nd</sup> PCR Amplification



### Diagnostic Test

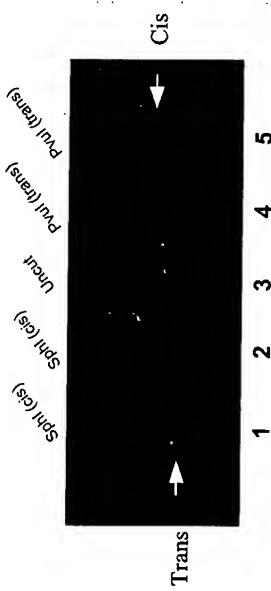
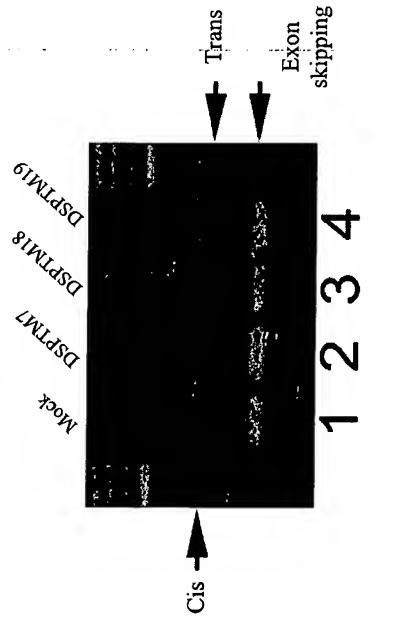


Figure 6A

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## Proof-of-principle of SMART using synthetic double splicing PTM RNA in stable cells

### 2nd PCR Amplification



### DSPTM18 and 19 (HCG targeted)

#### Methods

Transfect DSHCGT1.1 stable cells with DSPTM7, DSPTM18 and DSPTM19 *in vitro* transcribed, gel purified RNA (2:5:5.0 µg)

Isolate total RNA, cDNA synthesis (Lac6R), PCR amplification (20 cycles, K1-1F + Lac6R), digest with *Sph* I + *Dde* I (*cis*-specific) at 37°C/ON

Purify double *trans*-spliced product using Biotin-Lac21R probe

PCR amplify the captured *trans*-spliced product (K1-2F + Lac6R).  
Expected products: *cis*- 260bp; *trans*- 220 bp

Sequence to confirm the accuracy of double *trans*-splicing

Figure 6B

202 T 20 ° 820 S < DIDT

Accuracy of double trans-splicing of synthetic PIM RNA in 293T cells

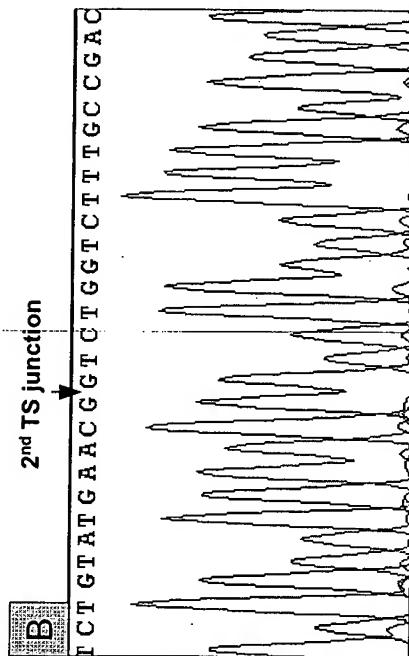
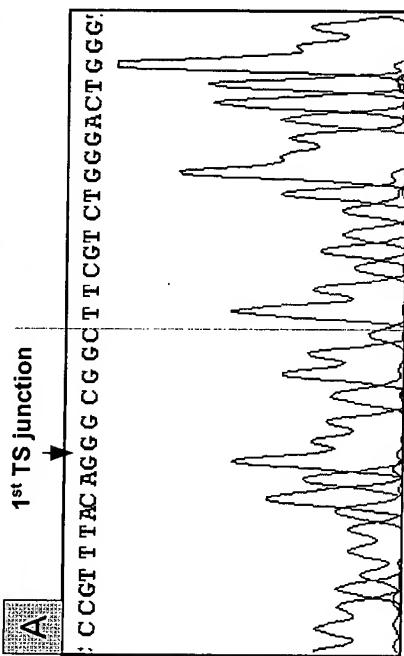


Figure 6C

## Restoration of $\beta$ -gal function through RNA transfection in 293T cells (Proof-of-concept for SMART RNA Therapeutics!!) Synthetic RNA, Double trans-splicing

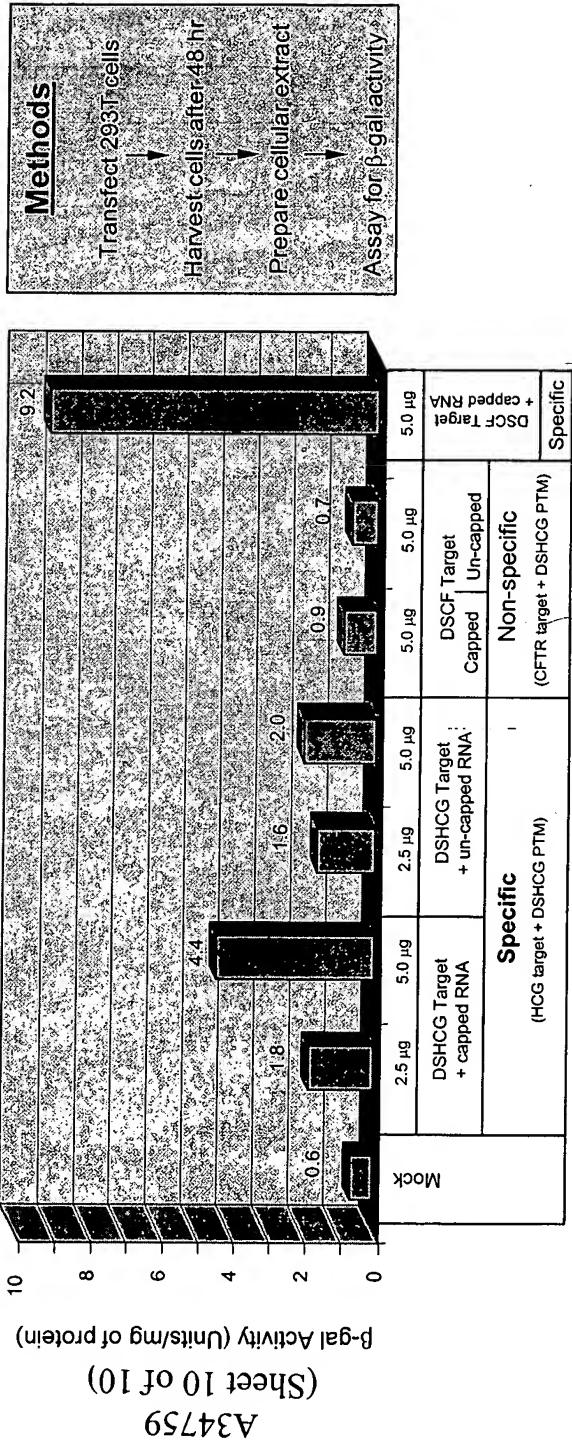


Figure 7